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 NEWS  5  NOV 30   PHAR reloaded with additional data
 NEWS  6  DEC 01   LISA now available on STN
 NEWS  7  DEC 09   12 databases to be removed from STN on December 31, 2004
 NEWS  8  DEC 15   MEDLINE update schedule for December 2004
 NEWS  9  DEC 17   ELCOM reloaded; updating to resume; current-awareness
                   alerts (SDIs) affected
 NEWS  10 DEC 17   COMPUAB reloaded; updating to resume; current-awareness
                   alerts (SDIs) affected
 NEWS  11 DEC 17   SOLIDSTATE reloaded; updating to resume; current-awareness
                   alerts (SDIs) affected
 NEWS  12 DEC 17   CERAB reloaded; updating to resume; current-awareness
                   alerts (SDIs) affected
 NEWS  13 DEC 17   THREE NEW FIELDS ADDED TO IFIPAT/IFIUDB/IFICDB

 NEWS EXPRESS  OCTOBER 29 CURRENT WINDOWS VERSION IS V7.01A, CURRENT
               MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP),
               AND CURRENT DISCOVER FILE IS DATED 11 AUGUST 2004
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FILE 'HOME' ENTERED AT 08:50:42 ON 27 DEC 2004

=> file medline embase biosis scisearch caplus
COST IN U.S. DOLLARS                            SINCE FILE      TOTAL
                                                   ENTRY    SESSION
FULL ESTIMATED COST                                 0.21       0.21

FILE 'MEDLINE' ENTERED AT 08:50:58 ON 27 DEC 2004

FILE 'EMBASE' ENTERED AT 08:50:58 ON 27 DEC 2004
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FILE 'BIOSIS' ENTERED AT 08:50:58 ON 27 DEC 2004

FILE 'SCISEARCH' ENTERED AT 08:50:58 ON 27 DEC 2004

FILE 'CAPLUS' ENTERED AT 08:50:58 ON 27 DEC 2004

=> s phOx antibod?
L1 78 PHOX ANTIBOD?

=> s l1 and single chain
L2 8 L1 AND SINGLE CHAIN

=> dup remove l2
PROCESSING COMPLETED FOR L2
L3 3 DUP REMOVE L2 (5 DUPLICATES REMOVED)

=> d l3 1-3 cbib abs

L3 ANSWER 1 OF 3 CAPLUS COPYRIGHT 2004 ACS on STN
2001:315197 Document No. 136:64863 A novel retroviral vector that allows the magnetic selection of infected cells. Zhang, J.; Sapp, C. M. (Department of Microbiology and Immunology, Markey Cancer Center, University of Kentucky, Lexington, KY, 40536-0096, USA). Journal of Virological Methods, 94(1-2), 1-6 (English) 2001. CODEN: JVMEDH. ISSN: 0166-0934. Publisher: Elsevier Science B.V..

AB Retroviral vectors are used widely in research and are also being designed for use in gene therapy trials. In practice, these vectors usually contain a marker gene, which is often a drug selection gene. In this report, a novel retroviral vector has been constructed which contains a gene that allows selection for infected cells by a magnet. This gene is a **single-chain** antibody (sFv) to a specific hapten mol. 4-ethoxymethylene-2-phenyl-2-oxazolin-5-one (phOx). sFv specific for phOx is displayed on the surface of infected cells. This feature allows binding to phOx-BSA coated magnetic beads which are used to isolate the infected cells.

L3 ANSWER 2 OF 3 MEDLINE on STN DUPLICATE 1
93372090. PubMed ID: 8364031. Phage display and selection of a site-directed randomized **single-chain** antibody Fv fragment for its affinity improvement. Riechmann L; Weill M. (MRC Laboratory of Molecular Biology, Cambridge, UK.) Biochemistry, (1993 Aug 31) 32 (34) 8848-55. Journal code: 0370623. ISSN: 0006-2960. Pub. country: United States. Language: English.

AB The affinity of an antibody Fv fragment was improved by semirational design involving site-directed randomization and phage display. On the basis of the predicted model of an anti-2-phenyloxazol-5-one (**phOx**) **antibody** Fv fragment, into which the ligand was inserted with the help of nuclear Overhauser enhancement (NOE) data, residues close to the hapten were identified. Seven of these residues in the third hypervariable regions of light and heavy chains were randomized in polymerase chain reactions (PCR) using degenerate oligonucleotides. Resulting clones were expressed as **single-chain** Fv (scFV) fragments on the surface of filamentous phage and selected for binding to phOx-conjugated bovine serum albumin. Selected Fv fragments were analyzed for hapten affinity by fluorescence quenching, and several mutants with improved affinities were identified. Phage selection on the basis of binding was very successful when phage scFv mutants differed in affinity by at least a factor of 6. Smaller differences did not result in

predominant selection of the best binder. Combination of the two point mutations most crucial for improved hapten binding decreased the dissociation constant of the Fv for phOx 11-14-fold. Hapten binding of the improved Fv was analyzed in NOE experiments.

L3 ANSWER 3 OF 3 CAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 2
1992:405633 Document No. 117:5633 Original and artificial antibodies. Olson, Edwin S. (Univers. Fuel Dev. Assoc., Inc., Grand Forks, ND, 58201, USA). Nature (London, United Kingdom), 357(6375), 201-2 (English) 1992. CODEN: NATUAS. ISSN: 0028-0836.
AB Hybridomas immortalize B cells by cell fusion. The monoclonal antibodies derived by this method are made by an original pair of heavy (H) and light (L) chains. The situation is different in the case of antibodies derived by PCR cloning of the variable (V) genes followed by their expression in bacteria. Here, the authors compared anti-2-phenyloxazolone (**phOx**) **antibodies** obtained by hybridomas with the already described **single chain** Fvs made with mRNA from the same pool of spleen cells. The random combinatorial library (containing 2 + 105 VH and Vk gene pairs assembled and expressed on a filamentous phage) had been selected on a phOx affinity column and clones ranked by ELISA. One of these bound phOx with a Kd = 10-8 mol-1. From the hybridomas derived from 5 + 107 spleen cells, 43 positives were obtained. These were similarly ranked by ELISA and several clones among the strong and medium binders were characterized further. One of these clones (HD11.4) bound phOx with a Kd = 2.7 + 10-9 mol-1. Thus, although both methods produce good binders, the best was isolated from a hybridoma. The gene combination of this antibody was not obtained from the random combinatorial library, and nor were other gene combinations typical of the anti-phOx response.

=> s sFv targeting
L4 0 SFV TARGETING

=> s receptor mediated targeting
L5 173 RECEPTOR MEDIATED TARGETING

=> s l5 and fluorescent probes
L6 5 L5 AND FLUORESCENT PROBES

=> dup remove l6
PROCESSING COMPLETED FOR L6
L7 1 DUP REMOVE L6 (4 DUPLICATES REMOVED)

=> d l7 cbib abs

L7 ANSWER 1 OF 1 MEDLINE on STN DUPLICATE 1
1999175123. PubMed ID: 10075643. **Receptor-mediated targeting** of **fluorescent probes** in living cells. Farinas J; Verkman A S. (Departments of Medicine and Physiology, Cardiovascular Research Institute, University of California, San Francisco, California 94143-0521, USA.. javier@itsa.ucsf.edu) . Journal of biological chemistry, (1999 Mar 19) 274 (12) 7603-6. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.
AB A strategy was developed to label specified sites in living cells with a wide selection of fluorescent or other probes and applied to study pH regulation in Golgi. cDNA transfection was used to target a single-chain antibody to a specified site such as an organelle lumen. The targeted antibody functioned as a high affinity receptor to trap cell-permeable hapten-fluorophore conjugates. Synthesized conjugates of a hapten (4-ethoxymethylene-2-phenyl-2-oxazolin-5-one, phOx) and **fluorescent probes** (Bodipy Fl, tetramethylrhodamine, fluorescein) were bound with high affinity (approximately 5 nM) and specific localization to the single-chain antibody expressed in the endoplasmic reticulum, Golgi, and plasma membrane of living Chinese

hamster ovary cells. Using the pH-sensitive phOx-fluorescein conjugate and ratio imaging microscopy, pH was measured in the lumen of Golgi (pH 6.25 +/- 0.06). Measurements of pH-dependent vacuolar H+/ATPase pump activity and H+ leak in Golgi provided direct evidence that resting Golgi pH is determined by balanced leak-pump kinetics rather than the inability of the H+/ATPase to pump against an electrochemical gradient. Like expression of the green fluorescent protein, the receptor-mediated fluorophore targeting approach permits specific intracellular fluorescence labeling. A significant advantage of the new approach is the ability to target chemical probes with custom-designed spectral and indicator properties.

=> s labeling living cell
L8 6 LABELING LIVING CELL

=> dup remove l8
PROCESSING COMPLETED FOR L8
L9 3 DUP REMOVE L8 (3 DUPLICATES REMOVED)

=> d l9 1-3 cbib abs

L9 ANSWER 1 OF 3 MEDLINE on STN DUPLICATE 1
2002062809. PubMed ID: 11788886. Characteristics of mitotic cell death induced by enediyne antibiotic lidamycin in human epithelial tumor cells. He Qi Yang; Liang Yun Yan; Wang Dai Shu; Li Dian Dong. (Institute of Medicinal Biotechnology, Chinese Academy of Medical Science and Peking Union Medical College, Beijing, P.R. China.) International journal of oncology, (2002 Feb) 20 (2) 261-6. Journal code: 9306042. ISSN: 1019-6439. Pub. country: Greece. Language: English.

AB Mitotic cell death, a different cell death mode from apoptosis, has been focused on in tumor therapy. It may involve the mechanism of highly potent cytotoxicities of enediyne antibiotics toward tumor cells. We describe the characteristics of mitotic cell death induced by enediyne antibiotic lidamycin at low concentrations (0.01-1 nM), in the human hepatoma BEL-7402 cells and human breast carcinoma MCF-7 cells. The cells exerting mitotic cell death showed retardation at G2+M phase, enlargement of cell volume and multinucleation, some of which were positive in senescence-associate beta-galactosidase staining. The multinucleated living cells did not show apoptotic features by co-staining with mitochondria-specific dye Mitosensor and DNA-specific dye Hoechst 33342. The DNA polyploidy rather than <apoptotic sub-G1 peak> increased with incubation time for the lidamycin-treated BEL-7402 cells. The proliferation status of BEL-7402 cells was shown by flow cytometry after the cells were labeled with PKH-67, a fluorescent dye for **labeling living cells**, but the fluorescent intensity of the lidamycin-treated cells was little changed. The smear DNA pattern was detected in the multinucleated cells by agarose gel electrophoresis. The results provide the first evidence for elucidating the potent cytotoxicities of lidamycin toward tumor cells and further describing characteristics of mitotic cell death.

L9 ANSWER 2 OF 3 CAPLUS COPYRIGHT 2004 ACS on STN
2001:747653 Document No. 135:294038 Magnetic-responsive dendrimer composition and its use in magnetic resonance analysis. Bulte, Jeff W. M.; Douglas, Trevor (The Government of the United States of America, as Represented by the Secretary, Department of Health and Human Services, National Institutes of Heath, USA). PCT Int. Appl. WO 2001074406 A2 20011011, 66 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC,

ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US10387 20010330. PRIORITY: US 2000-PV193360 20000331.

AB A magnetic probe that includes magnetic-responsive metal (oxyhydr)oxide particles stabilized by a dendrimer is described. The magnetic probe may be a magnetic resonance (MR) contrast agent in the form of an aqueous suspension that includes iron oxide particles synthesized in the presence of a carboxy-terminated poly(amidoamine) dendrimer. Such magnetic probes are useful for **labeling living cells** to render the cells magnetic resonance sensitive by contacting the magnetic probe with the cells to be labeled and allowing the metal (oxyhydr)oxide-dendrimer composition to be internalized by the cell. The magnetic probes can be made by in situ oxidizing a metal ion-containing material in the presence of a dendrimer. An example is given of a probe prepared from a carboxy-terminated gen 4.5 dendrimer and (NH4)2Fe(SO4)2 using Me3NO as oxidant and sodium citrate as chelating agent for removal of non-bound Fe(II). Properties of the nanoparticles are described. The particles were used for labeling human and animal cells which can be transplanted and tracked in vivo by MRI.

L9 ANSWER 3 OF 3 CAPLUS COPYRIGHT 2004 ACS on STN

2001:135278 Document No. 135:207645 Two-color fluorescence labeling of early and mid-to-late replicating chromatin in living cells. Schermelleh, Lothar; Solovei, Irina; Zink, Daniele; Cremer, Thomas (Ludwig Maximilians University, Munich, Germany). Chromosome Research, 9(1), 77-80 (English) 2001. CODEN: CRRSEE. ISSN: 0967-3849. Publisher: Kluwer Academic Publishers.

AB The authors report a modified scratch-loading protocol called scratch replication labeling (SRL) for labeling chromatin in adherently growing cells. The protocol involves making scratches with a hypodermic needle tip through cells grown to subconfluency on glass coverslips. At the time of scratching, the cells are pulse-labeled in culture medium containing Cy3-dUTP and Cy5-dUTP or Cy5-dUTP and FITC-dUTP. More efficient labeling was obtained by synchronizing the cells at the G1/S transition. Labeled cells continued to undergo mitosis and sequential labeling allowed visualization of early and mid-to-late replicating chromatin. The SRL protocol was used to label SH-EP N14 neuroblastoma cells, HeLa cells, BHK cells and primary human fibroblasts.

```
=> s GFP fusion
L10       5504 GFP FUSION

=> s l10 and golgi
L11        284 L10 AND GOLGI

=> s l11 and single chain
L12          0 L11 AND SINGLE CHAIN

=> s l11 and phOx
L13          0 L11 AND PHOX

=> s l11 and antibody
L14         42 L11 AND ANTIBODY

=> s l14 and single chain
L15          0 L14 AND SINGLE CHAIN

=> dup remove l14
PROCESSING COMPLETED FOR L14
L16          16 DUP REMOVE L14 (26 DUPLICATES REMOVED)

=> d l16 1-16 cbib abs

L16 ANSWER 1 OF 16 CAPLUS COPYRIGHT 2004 ACS on STN
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2003:513065 Document No. 140:177546 An improved chemical fixation method suitable for immunogold localization of green fluorescent protein in the **Golgi** apparatus of tobacco bright yellow (BY-2) cells. Follet-Gueye, Marie-Laure; Pagny, Sophie; Faye, Loic; Gomord, Veronique; Driouich, Azeddine (CNRS UMR 6037, IFRMP23, Universite de Rouen, UFR des Sciences, Centre Commun de Microscopie Electronique, Mont-Saint-Aignan, Fr.). Journal of Histochemistry and Cytochemistry, 51(7), 931-940 (English) 2003. CODEN: JHCYAS. ISSN: 0022-1554. Publisher: Histochemical Society, Inc..

AB In plant systems, the green fluorescent protein (GFP) is increasingly used as a marker to study dynamics of the secretory apparatus using fluorescence microscopy. The purpose of this study was to immunogold localize the GFP, at the electron microscopic level, in a line of tobacco BY-2-cultured cells, expressing a GFP-tagged **Golgi** glycosyltransferase. To this end we have developed a simple, one-step chemical fixation method that allow good structural preservation and specific labeling with anti-GFP **antibodies.** Using this method, we have been able to show that an N-glycan GFP-tagged xylosyltransferase is specifically associated with **Golgi** stacks of BY-2 transformed cells and is preferentially located in medial cisternae. As an alternative to cryofixation methods, such as high-pressure freezing, which requires specialized and expensive equipment not available in most labs., this method offers researchers the opportunity to investigate GFP-tagged proteins of the endomembrane system in tobacco BY-2 cells.

L16 ANSWER 2 OF 16 MEDLINE on STN

2003402588. PubMed ID: 12941522. Over-expression of wild-type and mutant HFE in a human melanocytic cell line reveals an intracellular bridge between MHC class I pathway and transferrin iron uptake. Fergelot Patricia; Orhant Magali; Thenie Agnes; Loyer Pascal; Ropert-Bouchet Martine; Lohyer Stephanie; Le Gall Jean-Yves; Mosser Jean. (UMR 6061 CNRS "Genetique et developpement", Faculte de Medecine, 2, avenue du Pr Leon-Bernard CS 34317, 35043 Rennes cedex, France.. patricia.fergelot@univ-rennes1.fr) . Biology of the cell / under the auspices of the European Cell Biology Organization, (2003 Jul) 95 (5) 243-55. Journal code: 8108529. ISSN: 0248-4900. Pub. country: France. Language: English.

AB Hereditary hemochromatosis (HH) is a frequent recessive disorder of iron metabolism characterised by systemic iron overload. In Northern Europe, more than 90% of HH patients are homozygous for a mis-sense mutation (C282Y) in the HFE1 gene product. The HFE protein is the heavy chain of a MHC class I-related molecule and associates with beta2 microglobulin and the transferrin receptor. Its precise roles in iron metabolism and in the pathophysiology of HH are still unclear. In order to identify the cellular processing of HFE, an important step towards the understanding of the function of the protein, we stably over-expressed the wild type and mutated forms fused to the Green Fluorescent Protein in a melanocytic MHC class I expressing cell line, the Mel Juso cell line. In wild type and mutant clones, the fusion proteins were not detected at the cell surface but only in the cytoplasm. Their sub-cellular localisation was determined by co-labelling of cells with organite-specific **antibodies** and confocal microscopy. HFE-GFP followed initially HLA class I intracellular processing but co-localised with transferrin in early endosomes without recycling at the cell surface. The C282Y-**GFP fusion** protein followed a different folding pathway to exit endoplasmic reticulum. Over-expression of the wild-type protein lead to a decrease in diferric transferrin uptake. Our model will be of use in the elucidation of the functional interaction between intracellular HFE and iron transporters transferrin/transferrin receptor complexes and Slc11A2 (also named N-Ramp2 or DMT1) in different endosomal compartments.

L16 ANSWER 3 OF 16 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN

2003:515000 Document No.: PREV200300512143. PROTEASOME - MEDIATED PAF - INDUCED DOWN - REGULATION OF PKCalpha IN ARPE - 19 CELLS. Faghiri, Z. [Reprint Author]; Bazan, N. G. [Reprint Author]. Neuroscience

Center/Ophthalmology, LSU Health Sciences Center, New Orleans, LA, USA. ARVO Annual Meeting Abstract Search and Program Planner, (2003) Vol. 2003, pp. Abstract No. 389. cd-rom.
Meeting Info.: Annual Meeting of the Association for Research in Vision and Ophthalmology. Fort Lauderdale, FL, USA. May 04-08, 2003. Association for Research in Vision and Ophthalmology.
Language: English.

AB Purpose: Protein kinases C (PKC) are key signaling isozymes implicated in physiologic and pathophysiologic processes. PKC alpha, beta, and delta are expressed in RPE. The messenger platelet-activating factor (PAF) has been implicated in RPE cell functions. Here we tested the hypothesis that PAF promotes translocation and down-regulation of PKCalpha in the RPE cell line ARPE-19. Methods: We studied expression and translocation patterns of PKCalpha-**GFP fusion** protein by fluorescence microscopy before and after cell stimulation by either PMA or PAF. PKCalpha-GFP accumulation in **Golgi** was examined by BODIPY TR ceramide staining, pretreatment of cells with a **Golgi** inhibitor, and immunostaining of cells with **antibodies** to p230 and GM130. Translocation of PKCalpha was confirmed by Western blot analysis of cytosolic and membrane fractions of ARPE-19. Down-regulation of PKCalpha and the role of proteasomes in its degradation were assayed by Western blot of whole-cell lysates of ARPE-19 stimulated with PAF with or without proteasome inhibitor lactacystin or MG-132. Results: PKCalpha was constitutively expressed in ARPE-19 cells with a rather diffuse cytoplasmic distribution. Upon stimulation with either PMA or PAF, PKCalpha was translocated to the plasma membrane and later accumulated within the **Golgi** as shown by BODIPY TR ceramide staining and immunostaining with **antibodies** to p230 and GM130. PAF antagonists BN50739, CV3988, or BN52021 prevented translocation of PKCalpha to **Golgi.** PLC inhibitor U-7312021 abolished PAF-induced PKCalpha-GFP translocation to the **Golgi**, indicating a G-protein-coupled receptor. **Golgi** inhibitor Brefeldin A prevented accumulation of PKCalpha in **Golgi** without affecting its membrane relocalization. PKCalpha activation is required for PAF-induced translocation to the **Golgi**, since pretreatment with PKC inhibitor calphostin prevented this translocation. Western blots of nontransfected ARPE-19 cells also showed translocation of constitutive PKCalpha from cytoplasm to membrane and down-regulation of its expression after stimulation by either PMA or PAF. Proteasome inhibitors prevented the depletion of PKCalpha in response to PAF or PMA. Conclusion: PAF induces activation and translocation of PKCalpha to the cytoplasmic membrane and **Golgi** through a G-protein-coupled receptor-specific mechanism involving the PLC-DAG pathway for PKCalpha activation. This activation and translocation leads to proteasome-dependent degradation of PKCalpha, suggesting the involvement of the ubiquitination pathway in degradation of PKCalpha in ARPE-19. These signaling events may be critical during RPE oxidative stress, when PAF production is enhanced. Supported by NIH EY05121

L16 ANSWER 4 OF 16 MEDLINE on STN DUPLICATE 1

2002424688. PubMed ID: 12181329. Casein kinase I regulates membrane binding by ARF GAP1. Yu Sidney; Roth Michael G. (Department of Biochemistry, University of Texas Southwestern Medical Center at Dallas, 75235-9038, USA.) Molecular biology of the cell, (2002 Aug) 13 (8) 2559-70. Journal code: 9201390. ISSN: 1059-1524. Pub. country: United States. Language: English.

AB ARF GAP1, a 415-amino acid GTPase activating protein (GAP) for ADP-ribosylation factor (ARF) contains an amino-terminal 115-amino acid catalytic domain and no other recognizable features. Amino acids 203-334 of ARF GAP1 were sufficient to target a **GFP-fusion** protein to **Golgi** membranes in vivo. When overexpressed in COS-1 cells, this protein domain inhibited protein transport between the ER and **Golgi** and, in vitro, competed with the full-length ARF GAP1 for binding to membranes. Membrane binding by ARF GAP1 in vitro was increased by a factor in cytosol and this increase was inhibited by IC261, an

inhibitor selective for casein kinase Idelta (CKIdelta), or when cytosol was treated with **antibody** to CKIdelta. The noncatalytic domain of ARF GAP1 was phosphorylated both in vivo and in vitro by CKI. IC261 blocked membrane binding by ARF GAP1 in vivo and inhibited protein transport in the early secretory pathway. Overexpression of a catalytically inactive CKIdelta also inhibited the binding of ARF GAP1 to membranes and interfered with protein transport. Thus, a CKI isoform is required for protein traffic through the early secretory pathway and can modulate the amount of ARF GAP1 that can bind to membranes.

L16 ANSWER 5 OF 16 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN

2001:713760 The Genuine Article (R) Number: 467QU. Mutant rab8 impairs docking and fusion of rhodopsin-bearing post-**Golgi** membranes and causes cell death of transgenic Xenopus rods. Moritz O L (Reprint); Tam B M; Hurd L L; Peranen J; Deretic D; Papermaster D S. Univ Connecticut, Ctr Hlth, Dept Neurosci, Farmington, CT 06032 USA (Reprint); Univ Helsinki, Inst Biotechnol, FIN-00014 Helsinki, Finland; Univ Michigan, Dept Ophthalmol & Visual Sci, Ann Arbor, MI 48105 USA; Univ Michigan, Dept Dev & Cell Biol, Ann Arbor, MI 48105 USA. MOLECULAR BIOLOGY OF THE CELL (AUG 2001) Vol. 12, No. 8, pp. 2341-2351. Publisher: AMER SOC CELL BIOLOGY. 8120 WOODMONT AVE, STE 750, BETHESDA, MD 20814-2755 USA. ISSN: 1059-1524. Pub. country: USA; Finland. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Rab8 is a GTPase involved in membrane trafficking. In photoreceptor cells, rab8 is proposed to participate in the late stages of delivery of rhodopsin-containing post-**Golgi** membranes to the plasma membrane near the base of the connecting cilium. To test the function of rab8 in vivo, we generated transgenic Xenopus laevis expressing wild-type, constitutively active (Q67L), and dominant negative (T22N) forms of canine rab8 in their rod photoreceptors as green fluorescent protein (**GFP**) **fusion** proteins. Wild-type and constitutively active GFP-rab8 proteins were primarily associated with **Golgi** and post-**Golgi** membranes, whereas the dominant negative protein was primarily cytoplasmic. Expression of wild-type GFP-rab8 had minimal effects on cell survival and intracellular structures. In contrast, GFP-rab8T22N caused rapid retinal degeneration. In surviving peripheral rods, tubulo-vesicular structures accumulated at the base of the connecting cilium. Expression of GFP-rab8Q67L induced a slower retinal degeneration in some tadpoles. Transgene effects were transmitted to F1 offspring. Expression of the GFP-rab8 fusion proteins appears to decrease the levels of endogenous rab8 protein. Our results demonstrate a role for rab8 in docking of post-**Golgi** membranes in rods, and constitute the first report of a transgenic X. laevis model of retinal degenerative disease.

L16 ANSWER 6 OF 16 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN

2001:302612 Document No.: PREV200100302612. RGS14, a regulator of heterotrimeric G-protein signaling localizes to centrosome and interacts with a centrosome protein, ninien. Cho, Hyeseou [Reprint author]; Kehrl, John H. [Reprint author]. NIH, 10 Center Dr., Bldg. 10 Rm. 11B08, Bethesda, MD, 20892, USA. FASEB Journal, (March 7, 2001) Vol. 15, No. 4, pp. A577. print.

Meeting Info.: Annual Meeting of the Federation of American Societies for Experimental Biology on Experimental Biology 2001. Orlando, Florida, USA. March 31-April 04, 2001.

CODEN: FAJOEC. ISSN: 0892-6638. Language: English.

AB RGS (Regulator of G protein Signaling) proteins are a family of over 20 proteins that negatively regulate heterotrimeric G-protein-coupled receptor signaling pathways by enhancing GTPase activities of Gialpha, Gqalpha and G12/13alpha. In addition to the RGS domain which is essential for the GTPase activating activity, many RGS proteins contain additional domains known to interact with a variety of signaling molecules such as 14-3-3, Rap1/2, RhoA, Gbeta5, GIPC, and polycystin. RGS14, a larger

member of the RGS family impairs Gialpha- and G13alpha-mediated signaling pathways and is strongly expressed in lymphocytes. To search for additional RGS14 functions, we performed a yeast 2-hybrid screen using a human spleen library with an RGS14 bait. We identified the human centrosome protein, ninein as well as several cytoskeletal and **Golgi** proteins. Using an RGS14 specific **antibody** we found that in some cells endogenous RGS14 localized within perinuclear dot-like structures, very suggestive of a centrosome location. Transfection of an RGS14-**GFP fusion** protein provided additional evidence for a centrosomal location. We are testing whether cell cycle position may explain the variable localization of RGS14 to centrosomes. Interestingly, the 130-amino acid region necessary for binding of RGS14 to small GTPases, Rap1/2 is sufficient to localize RGS14 to centrosomes. This suggests the possible involvement of a small GTPase in this process. Currently, we are mapping the interaction site between RGS14 and ninein as well as studying their co-localization by confocal microscopy. These results indicate that RGS14 likely has an important role in centrosome function.

L16 ANSWER 7 OF 16 MEDLINE on STN DUPLICATE 2

2002002963. PubMed ID: 11749975. **Golgi** retention of human protein NEFA is mediated by its N-terminal Leu/Ile-rich region. Nesselhut J; Jurgan U; Onken E; Gotz H; Barnikol H U; Hirschfeld G; Barnikol-Watanabe S; Hilschmann N. (Department of Immunochemistry, Max-Planck-Institute for Experimental Medicine, Hermann-Rein Str. 3, D-37075 Gottingen, Germany.) FEBS letters, (2001 Dec 14) 509 (3) 469-75. Journal code: 0155157. ISSN: 0014-5793. Pub. country: Netherlands. Language: English.

AB The subcellular localization of the human Ca(2+)-binding EF-hand/leucine zipper protein NEFA was studied in HeLa cells by immunofluorescence microscopy. Double immunostaining using mouse anti-NEFA monoclonal **antibody** 1H8D12 and rabbit anti-ERD2 polyclonal **antibody** proved that NEFA is localized in the **Golgi** apparatus. The result was confirmed by the expression of NEFA-green fluorescent protein (**GFP**) **fusion** protein in the **Golgi** in the same cell line. Cycloheximide treatment proved NEFA to be a **Golgi** -resident protein. Seven NEFA deletion mutants were constructed to ascertain the peptide region relevant for **Golgi** retention. The expression of each NEFA-GFP variant was detected by fluorescence microscopy and immunoblotting. Only the DeltaN mutant, lacking the N-terminal Leu/Ile-rich region, failed to be retained in the **Golgi** after cycloheximide treatment. The other six deletion mutants in which either the basic region, the complete EF-hand pair domain, the two EF-hand motifs separately, the leucine zipper and the leucine zipper plus the C-terminal region is deleted, were localized to the **Golgi.** The peptide sequence within the Leu/Ile-rich region is discussed as a novel **Golgi** retention motif.

L16 ANSWER 8 OF 16 MEDLINE on STN

2001141394. PubMed ID: 11163790. Novel type Arabidopsis thaliana H(+)-PPase is localized to the **Golgi** apparatus. Mitsuda N; Enami K; Nakata M; Takeyasu K; Sato M H. (Graduate School of Human and Environmental Studies, Kyoto University, Kyoto, Japan.) FEBS letters, (2001 Jan 12,) 488 (1-2) 29-33. Journal code: 0155157. ISSN: 0014-5793. Pub. country: Netherlands. Language: English.

AB Vacuolar H(+)-PPase, a membrane bound proton-translocating pyrophosphatase found in various species including plants, some protozoan and prokaryotes, has been demonstrated to be localized to the vacuolar membrane in plants. Using a GUS reporter system and a green fluorescent protein (**GFP**) **fusion** protein, we investigated the tissue distribution and the subcellular localization, respectively, of a novel type H(+)-PPase encoded by AVP2/AVPL1 identified in the Arabidopsis thaliana genome. We showed that AVP2/AVPL1 is highly expressed at the trichome and the filament of stamen. Furthermore, the fluorescence of GFP-tagged AVP2/AVPL1 showed small dot-like structures that were observed throughout

the cytoplasm of various Arabidopsis cells under a fluorescent microscope. The distribution of this dot-like fluorescent pattern was apparently affected by a treatment with brefeldin A. Moreover, we demonstrated that most dot-like fluorescent structures colocalized with a **Golgi** resident protein. These findings suggest that this novel type H(+)-PPase resides on the **Golgi** apparatus rather than the vacuolar membrane.

L16 ANSWER 9 OF 16 CAPLUS COPYRIGHT 2004 ACS on STN
2000:736740 Document No. 134:293444 Parkin gene and parkin protein; a key to understand nigral degeneration. Hattori, Nobutaka; Shimura, Hideki; Kubo, Shinichiro; Mizuno, Yoshikuni (Department of Neurology, Juntendo University School of Medicine, Hongo, Bunkyo-ku, Tokyo, 113-8421, Japan). Shinkei Kenkyu no Shinpo, 44(4), 555-566 (Japanese) 2000. CODEN: SKNSAF. ISSN: 0001-8724. Publisher: Igaku Shoin Ltd..

AB A review with 27 refs. In most patients with Parkinson's disease (PD), the contribution of genetic factors as well as environmental factors remains to be elucidated. However, it has become clear that genetic factors contribute to the pathogenesis of Parkinson's disease after identification of the distinct genetic loci for certain forms of familial Parkinson's disease and parkinsonism. The gene for an autosomal dominant familial form of Parkinson's disease was caused by mutations of the alpha-synuclein gene, and the authors recently identified the novel large gene "parkin" responsible for an autosomal recessive form of familial parkinsonism (AR-JP). AR-JP is a distinct clin. and genetic entity characterized by early onset before 40 yr, mild dystonia, diurnal fluctuation, spontaneous improvement of movement of disability after sleep or nap, a good response to levodopa, and less frequent resting tremor compared with sporadic Parkinson's disease. Pathol. changes in this form revealed selective degeneration of the pigmented neurons in the substantia nigra and locus coeruleus but no Lewy bodies were found. The parkin gene contains 12 exons spanning over 1 mega base and encodes a novel protein of 465 amino acids, mol. weight about 52 k dalton. The parkin gene is mildly homologous to ubiquitin at the N-terminal portion and has a RING finger like motif at the C-terminal portion. The authors found variable different homozygous deletions involving exons 3, 4, 5, 3 to 4, 3 to 5, 3 to 7, 6 to 7 in AR-JP families from Japan. In addition to exonic deletions, the authors identified a one base deletion in exon 5 in three AR-JP families. Furthermore, the authors identified point mutations in exon 6, and 8 in two Turkish families. Recently three papers appeared in the literature reporting the presence of mutations in parkin gene in families from France, Algeria, Italy Germany, Portugal and Greek. Therefore, this type of familial parkinsonism appears to have a world wide. Although the authors have identified several mutations in parkin gene, characterization of its gene product, "Parkin protein" has not yet been established. To elucidate the mol. mechanism underlying the disease, the authors have analyzed the subcellular localization of the Parkin protein by immunohistochem. and immunoblotting studies on patients with AR-JP and sporadic PD using two **antibodies** and using a recombinant protein by fusing the Parkin protein and green fluorescent protein (GFP) in cell cultures. Parkin protein was absent in all regions of the brains of AR-JP patients. Parkin protein was not decreased in brains of sporadic PD patients. Parkin protein was located in both **Golgi** complex and cytosol. In addition **GFP fusion** protein was also co-localized with WGA, a lectin labeled the **Golgi** complex in COS-1 cells. Furthermore, NGF-treated PC12 cells showed punctuate fluorescence, most likely vesicles, in the neuronal process. Taken together, the Parkin protein may play a role in vesicular transport system in association with the **Golgi** complex. Although the exact nature of the Parkin protein resulting in AR-JP remains to be clarified, the present study provides important clues for understanding the mechanism of the single gene defect leading to selective nigral degeneration as well as for rescuing disease nigral neurons.

L16 ANSWER 10 OF 16 MEDLINE on STN DUPLICATE 3

2001353653. PubMed ID: 11229601. Golvesin-**GFP fusions** as distinct markers for **Golgi** and post-**Golgi** vesicles in Dictyostelium cells. Schneider N; Schwartz J M; Kohler J; Becker M; Schwarz H; Gerisch G. (Max-Planck-Institut fur Biochemie, Martinsried, Germany.) Biology of the cell / under the auspices of the European Cell Biology Organization, (2000 Oct) 92 (7) 495-511. Journal code: 8108529. ISSN: 0248-4900. Pub. country: France. Language: English.

AB Golvesin is a new protein associated with membranes of the **Golgi** apparatus and post-**Golgi** vesicles in Dictyostelium cells. An internal hydrophobic sequence of 24 amino-acid residues is responsible for anchoring golvesin to the membranes of these organelles. In an attempt to visualize organelle dynamics in vivo, we have used specific **antibody** and other labels to localize golvesin-green fluorescent protein (GFP) constructs to different cellular compartments. With a GFP tag at its N-terminus, golvesin shows the same localization as the untagged protein. It is transferred to two post-**Golgi** compartments, the endosomal and contractile vacuole systems. Endosomes are decorated with GFP-golvesin within less than 10 min of their internalisation, and keep the label during the acidic phase of the pathway. Blockage of the C-terminus with GFP causes entrapment of the protein in the **Golgi** apparatus, indicating that a free C-terminus is required for transfer of golvesin to any of the post-**Golgi** compartments. The C-terminally tagged golvesin proved to be a reliable **Golgi** marker in Dictyostelium cells revealing protrusion of **Golgi** tubules at peak velocities of 3 to 4 microm x s(-1). The fusion protein is retained in **Golgi** vesicles during mitosis, visualizing **Golgi** disassembly and reorganization in line with cytokinesis.

L16 ANSWER 11 OF 16 MEDLINE on STN DUPLICATE 4

2001024865. PubMed ID: 11020350. Intracellular Fas ligand expression causes Fas-mediated apoptosis in human prostate cancer cells resistant to monoclonal **antibody**-induced apoptosis. Hyer M L; Voelkel-Johnson C; Rubinchik S; Dong J; Norris J S. (Department of Microbiology and Immunology, Medical University of South Carolina, Charleston, South Carolina 29425, USA.) Molecular therapy : journal of the American Society of Gene Therapy, (2000 Oct) 2 (4) 348-58. Journal code: 100890581. ISSN: 1525-0016. Pub. country: United States. Language: English.

AB Several laboratories have attempted with little success to induce Fas-mediated apoptosis in prostate cancer (PCa) cells, using different external Fas agonists, i.e., anti-Fas **antibodies** and membrane-bound FasL. The present study confirms these earlier results using the anti-Fas **antibody** CH-11 in five human PCa cell lines (PPC-1, LNCaP, PC-3, TSU-Pr1, and DU145). However, intracellular murine FasL expression induced Fas-mediated apoptosis in all CH-11-resistant cell lines. Adenovirus (AdGFPFasL(TET)) was used to deliver a Murine FasL-**GFP fusion** gene into human PCa cells resulting in 70-98% apoptosis at 48 h as determined by the MTS assay. DU145 and PPC-1 cells treated with AdGFPFasL(TET) stained positive for the TUNEL assay, indicating that cell death was via apoptosis. Using immunofluorescent microscopy, Fas and GFPFasL colocalized to the same intracellular compartment. The anti-Fas neutralizing **antibody** ZB-4 was unable to block AdGFPFasL(TET)-mediated cell death, suggesting that intracellular FasL may ligate Fas within the **Golgi** and/or endoplasmic reticulum. This is the first evidence suggesting that these two molecules interact prior to cell surface presentation. Collectively, these findings indicate that intracellular GFPFasL expression is superior to CH-11 at inducing Fas-mediated apoptosis in human PCa cells and may allow use of AdGFPFasL(TET) for PCa gene therapy.

L16 ANSWER 12 OF 16 MEDLINE on STN DUPLICATE 5

1999412380. PubMed ID: 10482615. Analysis of receptor usage by ecotropic murine retroviruses, using green fluorescent protein-tagged cationic amino acid transporters. Masuda M; Kakushima N; Wilt S G; Ruscetti S K; Hoffman P M; Iwamoto A. (Department of Microbiology, Graduate School of Medicine,

University of Tokyo, Tokyo 113-0033, Japan.. mmasuda@m.u-tokyo.ac.jp) . Journal of virology, (1999 Oct) 73 (10) 8623-9. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Entry of ecotropic murine leukemia virus (MuLV) into host cells is initiated by interaction between the receptor-binding domain of the viral SU protein and the third extracellular domain (TED) of the receptor, cationic amino acid transporter 1 (CAT1). To study the molecular basis for the retrovirus-receptor interaction, mouse CAT1 (mCAT1) was expressed in human 293 cells as a fusion protein with jellyfish green fluorescent protein (GFP). Easily detected by fluorescence microscopy and immunoblot analysis with anti-GFP **antibodies**, the mCAT1-**GFP fusion** protein was expressed in an N-glycosylated form on the cell surface and in the **Golgi** apparatus, retaining the ecotropic receptor function. The system was applied to compare Friend MuLV (F-MuLV) and its neuropathogenic variant, PVC-211 MuLV, which exhibits a unique cellular tropism and host range, for the ability to use various CAT family members as a receptor. The results indicated that F-MuLV and PVC-211 MuLV could infect the cells expressing wild-type mCAT1 at comparable efficiencies and that rat CAT3, but not mCAT2, conferred a low but detectable level of susceptibility to F-MuLV and PVC-211 MuLV. The data also suggested that CAT proteins might be expressed in an oligomeric form. Further application of the system developed in this study may provide useful insights into the entry mechanism of ecotropic MuLV.

L16 ANSWER 13 OF 16 MEDLINE on STN DUPLICATE 6

1999400425. PubMed ID: 10471377. Targeting of green fluorescent protein expression to the cell surface. Simonova M; Weissleder R; Sergeyev N; Vilissova N; Bogdanov A Jr. (Center for Molecular Imaging Research, Massachusetts General Hospital, Building 149, 13th Street, Charlestown, Massachusetts, 02129, USA.) Biochemical and biophysical research communications, (1999 Sep 7) 262 (3) 638-42. Journal code: 0372516. ISSN: 0006-291X. Pub. country: United States. Language: English.

AB We have previously reported on GPI-anchored fusion proteins that bind radioactive isotopes. We targeted their expression to the cell surface to obtain a marker protein detectable by nuclear and optical imaging (1, 2). Here we suggest a novel approach for targeting a model protein (GFP) to the exoplasmic surface of the plasma membrane. An expression vector (pcPEP-GFP) was constructed containing GFP cDNA fused with the fragment encoding the N-terminal cytoplasmic domain and signal peptide/membrane anchoring domain of the rabbit neutral endopeptidase (PEP-GFP). Flow cytometry showed green fluorescence in 45% of cells transfected with GFP and in 34% of cells transfected with PEP-GFP (24 h after transfection). Fluorescence microscopy of fixed cells stained with rhodaminated anti-GFP **antibodies** showed positive reaction only in the case of PEP-GFP-transfected cells indicating cell-surface expression. The PEP-**GFP fusion** protein was identified as a component of the light microsomal and **Golgi** fractions by immunoblotting.

L16 ANSWER 14 OF 16 MEDLINE on STN DUPLICATE 7

1999330190. PubMed ID: 10403396. The N-terminal 77 amino acids from tobacco N-acetylglucosaminyltransferase I are sufficient to retain a reporter protein in the **Golgi** apparatus of Nicotiana benthamiana cells. Essl D; Dirnberger D; Gomord V; Strasser R; Faye L; Glossl J; Steinkellner H. (Zentrum fur Angewandte Genetik, Universitat fur Bodenkultur-Wien, Austria.) FEBS letters, (1999 Jun 18) 453 (1-2) 169-73. Journal code: 0155157. ISSN: 0014-5793. Pub. country: Netherlands. Language: English.

AB In order to investigate sequences of tobacco N-acetylglucosaminyltransferase I (GnTI), involved in targeting to and retention in the plant **Golgi** apparatus the cytoplasmic transmembrane stem (CTS) region of the enzyme was cloned in frame with the cDNA of the green fluorescent protein (gfp) and subsequently transiently expressed in Nicotiana benthamiana plants using a tobacco mosaic virus (TMV) based expression vector. Confocal laser scanning microscopy showed

small fluorescent vesicular bodies in CTS-gfp expressing cells, while gfp alone expressed in control plants was uniformly distributed in the cytoplasm. The CTS-**gfp fusion** protein colocalised with immunolabelling observed by an **antibody** specific for the **Golgi** located plant Lewis a epitope. Furthermore, treatment with brefeldin A, a **Golgi** specific drug, resulted in the formation of large fluorescent vesiculated areas. These results strongly suggest a **Golgi** location for CTS-gfp and as a consequence our findings reveal that the N-terminal 77 amino acids of tobacco GnTI are sufficient to target to and to retain a reporter protein in the plant **Golgi** apparatus and that TMV based vectors are suitable vehicles for rapid delivery of recombinant proteins to the secretory pathway.

L16 ANSWER 15 OF 16 MEDLINE on STN

1999059801. PubMed ID: 9841901. Dual-color visualization of trans-**Golgi** network to plasma membrane traffic along microtubules in living cells. Toomre D; Keller P; White J; Olivo J C; Simons K. (Cell Biology/Biophysics Programme, European Molecular Biology Laboratory (EMBL), Meyerhofstrasse 1, Germany.) Journal of cell science, (1999 Jan) 112 (Pt 1) 21-33. Journal code: 0052457. ISSN: 0021-9533. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The mechanisms and carriers responsible for exocytic protein trafficking between the trans-**Golgi** network (TGN) and the plasma membrane remain unclear. To investigate the dynamics of TGN-to-plasma membrane traffic and role of the cytoskeleton in these processes we transfected cells with a **GFP-fusion** protein, vesicular stomatitis virus G protein tagged with GFP (VSVG3-GFP). After using temperature shifts to block VSVG3-GFP in the endoplasmic reticulum and subsequently accumulate it in the TGN, dynamics of TGN-to-plasma membrane transport were visualized in real time by confocal and video microscopy. Both small vesicles (<250 nm) and larger vesicular-tubular structures (>1.5 microm long) are used as transport containers (TCs). These TCs rapidly moved out of the **Golgi** along curvilinear paths with average speeds of approximately 0.7 micrometer/second. Automatic computer tracking objectively determined the dynamics of different carriers. Fission and fusion of TCs were observed, suggesting that these late exocytic processes are highly interactive. To directly determine the role of microtubules in post-**Golgi** traffic, rhodamine-tubulin was microinjected and both labeled cargo and microtubules were simultaneously visualized in living cells. These studies demonstrated that exocytic cargo moves along microtubule tracks and reveals that carriers are capable of switching between tracks.

L16 ANSWER 16 OF 16 MEDLINE on STN

1998192689. PubMed ID: 9524193. 1,25-Dihydroxy vitamin D3 and tri-iodothyronine stimulate the expression of a protein immunologically related to osteocalcin. Luegmayr E; Varga F; Glantschnig H; Fratzl-Zelman N; Rumpler M; Ellinger A; Klaushofer K. (Ludwig Boltzmann Institute of Osteology, Fourth Medical Department, Hanusch-Hospital, Vienna, Austria.) journal of histochemistry and cytochemistry : official journal of the Histochemistry Society, (1998 Apr) 46 (4) 477-86. Journal code: 9815334. ISSN: 0022-1554. Pub. country: United States. Language: English.

AB Osteocalcin (OC), a bone-specific protein, is a marker of late osteoblastic differentiation. Its expression is influenced by various growth factors and hormones. We investigated the effect of 1, 25-dihydroxy vitamin D3 (D3) and tri-iodothyronine (T3) on OC expression in osteoblast-like MC3T3-E1 cells. A heterologous OC green fluorescence protein (**GFP**) **fusion** vector was established and expressed to study possible effects on protein transport. Immunostaining of endogenous OC revealed a significant increase in the percentage of positive cells after D3 and T3 treatment. This was consistent for MC3T3-E1 cells as well as nonosteogenic NIH-3T3 and mammary carcinoma cells, but not for neuroblastoma cells. The perinuclear immunostaining corresponded to the NBD C6 ceramide **Golgi** staining. Conversely, we found a strong induction of OC in MC3T3-E1 cells at the mRNA and

protein levels only with T3 and not with D3. OC mRNA and protein expression was not detected in NIH fibroblasts. OC GFP transfection experiments indicate rapid transport and secretion of OC, because OC GFP was not found to be accumulated at intracellular compartments after hormone treatment. We conclude that the strong perinuclear immunostaining does not represent OC but a protein immunologically related to OC, as indicated by preabsorption experiments. The expression of this OC epitope-sharing protein is regulated by both D3 and T3 in the osteoblastic MC3T3-E1 and in nonosteogenic cells.

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L20 ANSWER 1 OF 7 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
2004219457 EMBASE Comparison of on-chip and off-chip microfluidic kinase assay formats. Dunne J.; Reardon H.; Trinh V.; Li E.; **Farinas J.**
. J. Farinas, Caliper Life Sciences Inc., 605 Fairchild Drive, Mountain View, CA 94043, United States. javier.farinas@calipertech.com. Assay and Drug Development Technologies 2/2 (121-129) 2004.
Refs: 14.
ISSN: 1540-658X. CODEN: ADDTAR. Pub. Country: United States. Language: English. Summary Language: English.
AB Kinases represent an important class of targets for pharmaceutical drug development. Microfluidic devices capable of running kinase assays with either an on-chip or an off-chip enzymatic reaction have been developed. For the on-chip assay, reagent addition, mixing, enzymatic reaction, and electrophoretic separation and detection of substrate and product all take place in the channels of the microfluidic chip. For the off-chip assay, the reaction takes place in a microtiter plate, whereas the electrophoretic separation and detection of substrate and product take place in the channels of the chip. To probe differences between the on-chip and off-chip assays, a panel of commercially available kinase inhibitors was assayed at 10 μM against cyclic AMP-dependent protein kinase A, glycogen synthase kinase 3β, mitogen- and stress-activated protein kinase, and Akt1 using both the off-chip and on-chip assays. Good correlation was observed between inhibition measured by the two methods, with most of the differences in measured inhibition being attributed to compound solubility and enzyme concentration effects. Microfluidic devices represent an attractive platform for kinase assays due to high data quality and the possibility of on-chip assay integration, leading to reagent and labor savings. .COPYRGT. Mary Ann Liebert, Inc.

L20 ANSWER 2 OF 7 CAPLUS COPYRIGHT 2004 ACS on STN
1999:659579 Document No. 131:283619 Methods and reagents for **targeting** organic compounds to selected cellular locations. **Farinas, Javier** (The Regents of the University of California, USA). PCT Int. Appl. WO 9951986 A1 19991014, 69 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN,

YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US7847 19990408. PRIORITY: US 1998-81118 19980408; US 1998-81340 19980409.

AB The present invention provides methods and reagents for **targeting** probes to selected cellular locations, through the expression of specific binding partners to that probe within the cell. In one embodiment, the probes may comprise spectroscopic probes that can be used in a method for localizing a specific binding partner within a cell, and for creating assays for post-translational activities. The invention allows the monitoring of the location of such intracellular specific binding partners over time and in response to stimuli, such as test chems. The spectroscopic probes can be used for screening a test chemical for activity. The present invention also includes cells and transgenic organisms comprising the intracellular specific binding partner, wherein the specific binding partner can bind with the spectroscopic probe/ligand conjugate. CHO cells were transfected with cDNAs encoding single chain antibody (sFv) fusion products with a Golgi-**targeting** human β-1,4-galactosyltransferase fragment. The sFv bound to hapten 4-ethoxymethylene-2-phenyl-2-oxazolin-5-one (phOx)-fluorescein conjugate. The Golgi-targeted phOx-fluorescein was used to detect continuous changes in lumenal pH in individual cells.

L20 ANSWER 3 OF 7 MEDLINE on STN DUPLICATE 1

1999175123. PubMed ID: 10075643. Receptor-mediated **targeting** of fluorescent probes in living cells. **Farinas J**; Verkman A S. (Departments of Medicine and Physiology, Cardiovascular Research Institute, University of California, San Francisco, California 94143-0521, USA.. javier@itsa.ucsf.edu) . Journal of biological chemistry, (1999 Mar 19) 274 (12) 7603-6. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB A strategy was developed to label specified sites in living cells with a wide selection of fluorescent or other probes and applied to study pH regulation in Golgi. cDNA transfection was used to target a single-chain antibody to a specified site such as an organelle lumen. The targeted antibody functioned as a high affinity receptor to trap cell-permeable hapten-fluorophore conjugates. Synthesized conjugates of a hapten (4-ethoxymethylene-2-phenyl-2-oxazolin-5-one, phOx) and fluorescent probes (Bodipy Fl, tetramethylrhodamine, fluorescein) were bound with high affinity (approximately 5 nM) and specific localization to the single-chain antibody expressed in the endoplasmic reticulum, Golgi, and plasma membrane of living Chinese hamster ovary cells. Using the pH-sensitive phOx-fluorescein conjugate and ratio imaging microscopy, pH was measured in the lumen of Golgi (pH 6.25 +/- 0.06). Measurements of pH-dependent vacuolar H+/ATPase pump activity and H+ leak in Golgi provided direct evidence that resting Golgi pH is determined by balanced leak-pump kinetics rather than the inability of the H+/ATPase to pump against an electrochemical gradient. Like expression of the green fluorescent protein, the receptor-mediated fluorophore **targeting** approach permits specific intracellular fluorescence labeling. A significant advantage of the new approach is the ability to target chemical probes with custom-designed spectral and indicator properties.

L20 ANSWER 4 OF 7 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN

1998:906514 The Genuine Article (R) Number: 137GQ. Receptor mediated **targeting** of fluorescent indicators for measurement of organelle ionic composition in living cells. **Farinas J (Reprint)**; Verkman A S. UCSF, CVRI, SAN FRANCISCO, CA. MOLECULAR BIOLOGY OF THE CELL (NOV 1998) Vol. 9, Supp. [S], pp. 604-604. Publisher: AMER SOC CELL BIOLOGY. PUBL OFFICE, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814. ISSN: 1059-1524. Pub. country: USA. Language: English.

L20 ANSWER 5 OF 7 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN

DUPLICATE 2
1998:334910 Document No.: PREV199800334910. **Targeting** fluorescent indicators for measurement of organelle ionic composition in living cells. **Farinas, Javier**; Verkman, A. S.. CVRI, UCSF, San Francisco, CA, USA. Biophysical Journal, (Feb., 1998) Vol. 74, No. 2 PART 2, pp. A184. print.
Meeting Info.: Forty-second Annual Meeting of the Biophysical Society. Kansas City, Missouri, USA. February 22-26, 1998.
CODEN: BIOJAU. ISSN: 0006-3495. Language: English.

L20 ANSWER 6 OF 7 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN
1999:18158 Document No.: PREV199900018158. Receptor mediated **targeting** of fluorescent indicators for measurement of organelle ionic composition in living cells. **Farinas, Javier**; Verkman, A. S.. CVRI, UCSF, San Francisco, CA, USA. Molecular Biology of the Cell, (Nov., 1998) Vol. 9, No. SUPPL., pp. 104A. print.
Meeting Info.: 38th Annual Meeting of the American Society for Cell Biology. San Francisco, California, USA. December 12-16, 1998. American Society for Cell Biology.
CODEN: MBCEEV. ISSN: 1059-1524. Language: English.

L20 ANSWER 7 OF 7 MEDLINE on STN DUPLICATE 3
95365338. PubMed ID: 7543677. Constitutive and regulated membrane expression of aquaporin 1 and aquaporin 2 water channels in stably transfected LLC-PK1 epithelial cells. Katsura T; Verbavatz J M; **Farinas** J; Ma T; Ausiello D A; Verkman A S; Brown D. (Renal Unit, Massachusetts General Hospital, Boston 02114, USA.) Proceedings of the National Academy of Sciences of the United States of America, (1995 Aug 1) 92 (16) 7212-6. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.
AB The aquaporins (AQPs) are a family of homologous water-channel proteins that can be inserted into epithelial cell plasma membranes either constitutively (AQP1) or by regulated exocytosis following vasopressin stimulation (AQP2). LLC-PK1 porcine renal epithelial cells were stably transfected with cDNA encoding AQP2 (tagged with a C-terminal c-Myc epitope) or rat kidney AQP1 cDNA in an expression vector containing a cytomegalovirus promoter. Immunofluorescence staining revealed that AQP1 was mainly localized to the plasma membrane, whereas AQP2 was predominantly located on intracellular vesicles. After treatment with vasopressin or forskolin for 10 min, AQP2 was relocated to the plasma membrane, indicating that this relocation was induced by cAMP. The location of AQP1 did not change. The basal water permeability of AQP1-transfected cells was 2-fold greater than that of nontransfected cells, whereas the permeability of AQP2-transfected cells increased significantly only after vasopressin treatment. Endocytotic uptake of fluorescein isothiocyanate-coupled dextran was stimulated 6-fold by vasopressin in AQP2-transfected cells but was only slightly increased in wild-type or AQP1-transfected cells. This vasopressin-induced endocytosis was inhibited in low-K+ medium, which selectively affects clathrin-mediated endocytosis. These water channel-transfected cells represent an in vitro system that will allow the detailed dissection of mechanisms involved in the processing, **targeting**, and trafficking of proteins via constitutive versus regulated intracellular transport pathways.

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